Sponsors

University of Minnesota
College of Veterinary Medicine

College of Agricultural, Food and Environmental Sciences

Extension Service

Swine Center

Editors
W. Christopher Scruton
Stephen Claas

Layout
David Brown

Logo Design
Ruth Cronje, and Jan Swanson;
based on the original design by Dr. Robert Dunlop

Cover Design
Shawn Welch

Defining resistance

Mike Apley, DVM, PhD, DACVCP
Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, Iowa

Measuring susceptibility and resistance

We throw around the terms "susceptible" and "resistant" quite often when discussing the interaction of antimicrobials with bacteria that cause disease. While the public may not understand what makes something "resistant," it sure sounds bad. To define "resistant," we must start with two key questions:

- How is antimicrobial susceptibility testing conducted?
- How is antimicrobial susceptibility testing interpreted?

There are two primary methods for bacterial susceptibility profile determination that veterinary diagnostic laboratories use today.

Microwell dilution method

This system uses a plate with wells that contain different concentrations of the selected antimicrobials. Ideally we would have wells for each antimicrobial at 1:2 dilution intervals across an appropriate range to accurately evaluate the minimal inhibitory concentration (MIC) of each compound for the pathogen. However, cost prohibits this technique from routine use, so testing often focuses on breakpoints that are selected based on reported serum/plasma pharmacokinetic properties, clinical data, and microbiological data for antimicrobials in the species of interest. For example, commonly used breakpoints for tetracycline are 4 and 8 mg/mL.

- A pathogen growing in neither of the wells would be considered susceptible.
- A pathogen growing only in the 4 mg/mL well would be considered intermediately susceptible.
- A pathogen growing in both wells would be considered resistant.

However, these breakpoints for the tetracyclines have not been validated for clinical applications in veterinary medicine but have been selected by the National Committee for Clinical Laboratory Standards Veterinary Antimicrobial Susceptibility Testing Subcommittee (NCCLS/VAST) from breakpoints developed in human medicine (see below) as reasonable for use in veterinary medicine. It is important to know the validation status of breakpoints used for susceptibility testing.

Kirby-Bauer (disk diffusion)

In this method, a paper disk containing the antimicrobial is placed on an agar plate that has been inoculated with the pathogen. The plate is incubated and the zones of inhibition (absence of any visible bacterial growth) are measured surrounding the disks. The diameter of the zone is correlated back to serial-dilution concentrations used to set "susceptible," "intermediate," and "resistant" classifications for pathogens. This technique is obviously heavily dependent on quality control. Depth and contents of the agar, bacteria concentration in the inoculum, and antimicrobial contents of the disks must be closely controlled.

You have to have standards!

The tests described above must be conducted according to exacting standards. These standards may be found in the NCCLS publication M31-A2: *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals: Approved Standard* - Second Edition (ISBN 1-56238-461-9). This publication was prepared and is constantly updated by the VAST Subcommittee of the NCCLS through a consensus process. Copies of the current edition may be obtained from NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA. The NCCLS office may be reached at 610-688-0100, (fax) 610-699-0700, or on the web at Exoffice@nccls.org.

In addition to standards for conducting the tests, the NCCLS also provides standards for interpreting the tests as described below. Sometimes authors claim to have adhered to NCCLS interpretive criteria for "resistance." This doesn't mean much unless they also adhered to NCCLS standards for conducting the tests. Many of the publications are authored by people that have no idea whether the interpretive criteria they are citing have been validated for the application they are discussing.

Susceptible, intermediate, and resistant?

One point of confusion is the difference between an MIC and a breakpoint. An MIC is the concentration required

to inhibit growth *in vitro* (under standardized conditions) for a standardized time period. A breakpoint expands upon this concentration to predict clinical outcome for a specific pathogen, in a specific disease, in a specific species, given a specific regimen (dose, route, duration, frequency). Therefore, a breakpoint is a specific MIC at which we expect clinical success (susceptible) or clinical failure (resistant). More precise definitions of susceptible, intermediately susceptible, and resistant are described below.

The National Committee for Clinical Laboratory Standards Veterinary Antimicrobial Susceptibility Testing Subcommittee has approved veterinary-specific breakpoints for applications shown in **Table 1**. In each application, specific pathogens are indicated in M31-A2 for which the interpretive criteria are valid.

For other antimicrobials used in veterinary medicine, sponsors have not presented data on clinical trials, pharmacokinetics, pharmacodynamics, and pathogen susceptibility profiles to the NCCLS. In these cases, the committee may utilize available information and adapt breakpoints developed in human medicine. This is the case for penicillin G, ampicillin, tetracycline, potentiated sulfas, and the aminoglycosides. The take-home point is that, when discussing "resistance," you need to know whether the determination was made using a validated breakpoint (which we would expect to be more closely correlated with clinical outcome than an adapted breakpoint) or one that has been adapted from other applications.

It is also important to know exactly what the classifications of "susceptible," "intermediately susceptible," and "resistant" are intended to mean. The passages printed below are reproduced with permission from NCCLS.

Some definitions from M31-A2

2.8.2 Susceptible

This category implies that there is a high likelihood of a favorable clinical outcome when the drug is administered at label dosage, because of adequate pharmacodynamic parameters relative to the MIC of the causative organism.

2.8.3 Intermediate

This category provides a "buffer zone". This buffer zone should prevent small, uncontrolled technical factors from causing discrepancies in interpretations (e.g., a resistant organism being categorized as susceptible [termed a very major error], or a susceptible organism being categorized as resistant [termed a major error], especially for drugs with narrow pharmacotoxicity margins.

This category includes strains with MICs that approach or can exceed usually attainable blood or tissue levels (but do not have flexible labeling); and for which response rates can be lower than for strains in the "susceptible" category. These strains can be inhibited by attainable concentrations of certain antimicrobial agents:

In body sites, such as the urinary tracts, where drugs are physiologically concentrated (e.g., quinolones, b-lactams); and

Provided the drug has a wide pharmacotoxicity margin and is administered at maximal dosage (e.g., B-lactams).

If the organism is not susceptible to alternative clinically feasible drugs, if the site of infection is not one where the drug is concentrated, or if the high dose cannot be used, the test should be repeated.

Table 1: The National Committee for Clinical Laboratory Standards Veterinary Antimicrobial Susceptibility Testing Subcommittee has approved veterinary-specific breakpoints for these applications.

Antimicrobial	Species	Application
Ceftiofur	bovine, swine, equine	respiratory disease
Tilmicosin	bovine	respiratory disease
	swine	respiratory disease
Danofloxacin	bovine	respiratory disease
Difloxacin	canine	dermal, UTI
Marbofloxacin	feline	dermal
	canine	dermal, UTI
Orbifloxacin	feline	dermal
	canine	dermal, UTI
Enrofloxacin	chickens and turkeys	*P. multocida, E. coli*
	bovine	respiratory disease
	canine and feline	dermal, URI, UTI
Penicillin/novobiocin	bovine	mastitis
Florfenicol	bovine	respiratory disease
	swine	respiratory disease
Spectinomycin sulfate	bovine	bovine respiratory disease
Pirlimycin	bovine	mastitis
Clindamycin	canine	soft tissue infections
Tiamulin	swine	respiratory disease

2.8.5 Resistant
This category implies that there will not be a favorable outcome, because the achievable systemic concentrations of the agent will be lower than the MIC of the causative organism with normal dosage schedules and/or fall in the range or where specific microbial resistance mechanisms are likely (e.g., B-lactamases), and clinical efficacy has not been reliable in treatment studies.

Practitioners commonly receive susceptibility information from both standard dilution tests and disk diffusion tests. How do MICs and zone diameters relate?

3.1 Equivalent MIC Breakpoints
Disk diffusion zone diameters correlate inversely with MICs from standard dilution tests, usually broth microdilution. Table 2 [not reproduced here] lists the zone diameters and MIC breakpoints used for the interpretive guidelines. Zone diameters and MIC breakpoints are correlated based upon zone-diameter versus MIC regression, population distributions, pharmacokinetics, and clinical efficacy studies. However, the zone diameters may not correspond precisely to the listed MIC breakpoints due to differences in the methodologies and the original databases. Thus, the information provided in Table 2 cannot be used to convert zone diameters to absolute MIC values. See also Section 1.3"

What about susceptibility testing where NCCLS veterinary-specific interpretive criteria are not available? A distinction between veterinary-specific interpretive criteria and criteria adapted from human medicine is made in M31-A2. The "table 2" referred to in the quote is the table in M31-A2 reporting interpretive criteria.

2.8.1 MIC and Zone-Size Interpretive Criteria
Table 2 shows interpretive criteria for the sizes of the zones of inhibition for use with agar disk diffusion susceptibility tests and MIC breakpoints for use with dilution susceptibility tests. For those agents for which veterinary-specific interpretive criteria are not available, the use of these values in relation to veterinary bacterial isolates must be done with caution for three reasons. First, the value listed in the gray shaded areas listed in Table 2 were developed in human medicine by comparing zone diameters to MICs in broth or agar dilution tests and from population distributions of zones and/or MICs of known susceptible and resistant strains. Second, the MICs and correlated zone-size distributions were analyzed in relation to the clinical pharmacokinetics of the drug from normal dose-range schedules in humans. Third, the in vitro and pharmacologic data have been analyzed in relation to studies of clinical outcome of treatment of specific human pathogens.
Additionally, caution should be exercised in using the interpretive criteria listed in Table 2. These criteria apply to particular uses of the antimicrobial drugs in specific animal species. Extension of these data to other disease indications or other animal species may lead to an incorrect prediction of clinical outcome. Antimicrobial concentrations differ across regions of the body depending on the specific drug, route of administration , drug formulation, and the animal's metabolism, and these differences can profoundly affect clinical performance of the drug. Therefore, the subcommittee has listed only approved animal species and pathogens in Table 2 to define those conditions where interpretive criteria are known to be applicable . . .

Summary

When discussing "resistance" it is important to know whether the testing and interpretation were done according to NCCLS standards. It is also very important to know whether the interpretive breakpoints were validated specifically for the application for which "resistance" is being discussed. It is very important to recognize that an antimicrobial does not have a breakpoint that applies uniformly to all combinations of regimen, animal species, disease, and bacterial species. In fact, it is not uncommon for an antimicrobial to have multiple breakpoints for different applications.